

GRAPEVINE NECROTIC UNION, A NEWLY RECOGNIZED DISEASE OF UNKNOWN ETIOLOGY IN GRAPEVINES GRAFTED ON 110 RICHTER ROOTSTOCK IN CALIFORNIA

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SUMMARY

In Northern California, surveys of several vineyards planted to *Vitis vinifera* cv. Pinot noir (PN) clones 02A, 667, 777, and UCD 04 grafted onto the rootstock *V. berlandieri* x *V. rupestris* 110 Richter (110R) revealed 2 to 45% of vines showing solid red leaf canopies and two distinct disease stages, i.e., full canopied plants with normal grape clusters (designated as acute) or stunted shoots and straggly bunches (designated as chronic). In addition, the scion-rootstock junction of symptomatic grapevines had developed a necrotic union; hence, the name 'grapevine necrotic union' (GNU). Also, two white fruited cultivars, Chardonnay (clone 04, vineyard in Yolo county) and Pinot gris (clone 152, vineyard in Monterey county), exhibited GNU-associated symptoms, with an exception of pale yellowed leaves. A detailed survey, from 2004 to 2009, was made in a sub-block containing 664 grapevines (8 rows x 80-85 grapevines) in a PN 02A vineyard (planted in 1997) located in Sonoma county. The initial incidence of GNU was 2.1% in 2004 and rose to 21.9% by 2009 suggesting secondary spread. Molecular assays (RT-PCR and PCR) for grapevine viruses, phytoplasmas, and *Xylella fastidiosa*, failed to detect a putative causal agent. Likewise, bud-chip inoculations of test plants of Cabernet sauvignon scions on different rootstocks, including 110R, were inconclusive. However, several bench-grafts of collections of PN 02A, PN 91 and Chardonnay 04 onto 110R produced plants that developed GNU symptoms in a research plot. Based on the results obtained so far, GNU is being regarded as an emerging disease of unknown etiology in California vineyards.

Key words: red leaf, bench-graft assays, RT-PCR, grapevine, *Vitis*, Pinot noir.

INTRODUCTION

California is the major grape producer in the USA with 90% of all grapes harvested in 2009, 57% of which were for wine production (<http://www.nass.usda.gov/>). The diversity of grape-growing regions in this state requires a variety of rootstocks to meet various regional demands such as resistance to soil-borne pests and diseases, differences in soil types, and/or drought tolerance. In California, AXR#1 (*Vitis vinifera* 'Aramon' x *V. rupestris* 'Ganzin') was the rootstock of choice from the 1970s through the 1980s, a period of rapid vineyard expansion. But, in the mid-eighties, AXR#1 began to fail with the emergence of phylloxera biotype B, necessitating the use of other rootstocks (Granett *et al.*, 1985). Use of these rootstocks controlled the phylloxera biotype B, but infectious agents, latent in scions and in AXR#1, developed severe reactions in the form of graft incompatibility (Golino, 1992; Golino *et al.*, 2000; Uyemoto *et al.*, 2000, 2001).

Grapevines suffer from diseases caused by a diverse set of infectious agents. Among them, viruses play a significant role in affecting their overall performance. Leafroll disease, caused by grapevine leafroll-associated viruses, is recognized by its distinct reddening (in dark fruit varieties) or yellowing (light fruit varieties) of interveinal regions of the leaf blades with primary veins remaining green. Leafroll was described earlier as 'red leaf' by Goheen and Cook (1959). But, the term 'red leaf' is now used to describe leaf blades completely red in color (Namba *et al.*, 1991; Uyemoto *et al.*, 2001), which develop in leaves distal to trunks or canes as a general host response in red fruited varieties to some form of girdling caused by abiotic or biotic factors.

Some virus infections can lead to graft incompatibility as well as stem markings to cause red leaf disease on grapevines grown on specific rootstocks. As an example, vitiviruses associated with grapevine corky bark disease cause leaves to turn red in LN-33, due directly to vascular tissue degeneration of canes and trunks. In France and Italy, *Grapevine leafroll-associated virus 2* (GLRaV-2, family *Closteroviridae*) is associated with graft incompatibility of cv. Chardonnay accessions on the rootstock Kober 5BB (*V. berlandieri* x *V. riparia*)

(Greif *et al.*, 1995). Meanwhile in California, a red leaf and decline of grapevines was observed in field trials of cv. Redglobe table grape bench-grafted onto four rootstocks, namely, 3309 Couderec (*V. riparia* x *V. rupestris*), 1103 Paulsen (*V. berlandieri* x *V. rupestris*), Kober 5BB, and Teleki 5C (*V. berlandieri* x *V. riparia*). Eventually, a strain of GLRaV-2 (Rowhani *et al.*, 2000; Uyemoto *et al.*, 2000, 2001) was identified as the putative causal agent.

Herein, we report on a new grapevine disease called 'grapevine necrotic union' (GNU), an emerging threat to grapevines grafted onto 110 Richter (*V. berlandieri* x *V. rupestris*) in California vineyards.

MATERIALS AND METHODS

Vineyard surveys. Declining grapevines of cv Pinot noir (PN) clones 02A, 667, 777, and UCD 04 on the rootstock 110R, showing red canopies were examined for foliar symptoms and trunk disorders in several vineyards in Napa county. Later, surveys were extended to Monterey, Sonoma, and Yolo counties in 110R-rooted vineyards of PN clones and two white fruited cvs Chardonnay (clone 04) and Pinot gris (clone 152). Collections of diseased and healthy trunk unions were partially submerged in water, autoclaved (5 min; fast exhaust cycle), and bark was removed to expose the woody cylinders.

Annual surveys were done in a vineyard of PN 02A/110R (established in 1997, located in Sonoma county; hereafter referred to as Sonoma-1) from 2004 through to 2009 in a sub-block consisting of 8 rows x 80-85 grapevines (total 664 grapevines). Symptomatic grapevines were mapped and a few randomly selected plants examined for the presence of necrotic unions by removing a small bark patch at the graft unions. This vineyard site was selected because of its initial low disease incidence (2.1%) and block isolation, e.g., upper border adjacent to natural rangeland and lower perimeters encircled by a natural drainage ditch and access road. Cumulative numbers of diseased grapevines (new ones plus those previously identified) were used to determine the disease incidence and plotted using Microsoft Excel 2007.

Mechanical inoculations of herbaceous hosts. Attempts to determine etiological agents involved mechanical inoculations onto the following herbaceous indicators: *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus*, *Gomphrena globosa*, *Nicotiana benthamiana*, *N. clelandii*, *N. glutinosa*, and *N. occidentalis*. Young and mature leaves of several symptomatic and symptomless grapevines from vineyards Napa and Sonoma-1 were triturated in chilled 0.05 M sodium phosphate buffer containing 2% nicotine. Resulting extracts were rubbed onto

bentonite-dusted leaves with cotton tipped applicators. Buffered extracts of *N. benthamiana* plants infected with *Cucumber mosaic virus* (CMV) and healthy tobacco extracts served as positive and negative controls. Six to eight plants were used for each host tested. Inoculated plants were held in a greenhouse and monitored over several weeks for symptoms of virus infections.

Bud-chip grafts and bench-grafts on woody indicators. In August 2005, mature canes from nine symptomatic (sources exhibiting acute or chronic symptoms) and one asymptomatic grapevine of PN 02A were collected from vineyard Sonoma-1 and bud-chip grafted onto test plants of 2nd-leaf Cabernet sauvignon 08 (CS-8) propagated on 10 different rootstocks, i.e. Freedom and Harmony (both are hybrids of open pollinated selections that include parents of multiple speciation, i.e., *V. champinii*, *V. labrusca*, *V. riparia*, *V. solonis*, and *V. vinifera*), Kober 5BB (*V. berlandieri* x *V. riparia*), SO4 (*V. berlandieri* x *V. riparia*), Teleki 5C (*V. berlandieri* x *V. riparia*), 101-14 Mgt (*V. riparia* x *V. rupestris*), 110R and 1103P (both *V. riparia* x *V. rupestris*), 1616C (*V. solonis* x *V. riparia*), and 3309C (*V. riparia* x *V. rupestris*). A year later, another collection of 10 diseased sources with either acute or chronic symptoms was made and was bud-chip inoculated onto test plants of cv. Chardonnay 04 on rootstocks Freedom, 110R, 3309C, 5BB, 5C, and 1616C. Six bud-chips per collection were grafted onto test plants maintained in the field. After 30 days incubation, bud-chips were read for viability and test plants monitored for expression of symptoms for three growing seasons.

Concomitantly, a second assay procedure involved bench-grafts of dormant cuttings of scion collections directly onto dormant cuttings of rootstocks 110R and 3309C. For each collection, six two-bud scion pieces, ca. 15 cm in length, were inserted onto six cuttings of each rootstock, which were individually labeled, then layered in moist peat moss-perlite mixture in plastic containers, and stored for 4-6 weeks in a heated (27°C) room to promote development of callus tissues and initiation of roots. Rooted bench-grafts were rinsed in water and scion portions dipped in a molten wax bath, cooled immediately in an ice bath, and transplanted into carton sleeves (5 cm square x 25 cm long) filled with a moist peat moss-perlite mixture. Cartons were held in trays, tops of bench-grafts overlaid with newspaper, and placed on heat pads in the greenhouse. After 60 days, five plants per collection were transplanted in the field, shoots were trained onto redwood posts, and monitored for disease symptoms.

Dormant canes for the above bench-graft assays involved a collection of 60 grapevines obtained during February 2006. The collection consisted of 11 symptomatic and one symptomless grapevines of PN 02A from vineyard Sonoma-1 and 48 symptomless grapevines from an University of California-Davis (UCD) vineyard

comprised of 11 collections of PN 02A, two each of PN 71, 72, 91, 93, and 667, and 27 of cv. Chardonnay 04. The grapevines in vineyard UCD were either own-rooted or grown on rootstock Kober 5BB and devoid of GNU symptoms.

Molecular assays for grapevine viruses, phytoplasmas, and *Xylella* sp. During 2006 and 2009, total nucleic acid (TNA) preparations were made using 0.2 g of bark scrapings from mature canes of diseased and asymptomatic grapevines or own-rooted plants coming from vineyards Napa, Sonoma-1, and UCD. Bark tissues were extracted with the RNeasy Plant mini kit (Qiagen, USA) without DNase treatment.

RT-PCR assays were performed using several primers for grapevine viruses and reaction conditions as described by Osman *et al.* (2008). The grapevine viruses tested included members in the family *Closteroviridae*: *Grapevine leafroll-associated virus 1* (GLRaV-1), -2, -3, -4, -5, -7, -9, *Grapevine leafroll-associated Carnelian virus* (Abou Ghanem-Sabanadzovic *et al.*, 2010) and GLRaV-2 strain red globe (GLRaV-2RG); members in the family *Betaflexiviridae*: *Grapevine rupestris stem pitting associated virus* (GRSPaV) and GRSPaV-Syrah strain, *Grapevine virus A* (GVA), *Grapevine virus B* (GVB), and *Grapevine virus D* (GVD); the family *Secoviridae*: *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Tobacco ringspot virus* (TRSV), and *Tomato ringspot virus* (ToRSV); and the family *Tymoviridae*: *Grapevine fleck virus* (GFkV), *Grapevine rupestris vein feathering virus* (GRVfV), and *Grapevine Syrah virus-1* (GSyV-1). Primers for ArMV, GFLV, GLRaV-7, GRSPaV-Syrah, GRVfV, GSyV-1, GVD, and ToRSV were from previous studies (Al Rwahnih *et al.*, 2009; Lima *et al.*, 2006; Osman and Rowhani, 2006; Osman *et al.*, 2008; Turturo *et al.*, 2000). Sources for known viruses and healthy grapevines were own-rooted plants maintained in a greenhouse.

PCR assays were also used to detect phytoplasmas with universal primers designed by Gundersen and Lee (1996), Lee *et al.* (1993), and Lorenz *et al.* (1995) and by Minsavage *et al.* (1994) for *Xylella fastidiosa*. *Vinca rosea* plants infected with Cherry X disease phytoplasma and a stored bacterial culture of *X. fastidiosa* were used as positive controls. Presence of amplified products was assessed by agarose gel electrophoresis.

Besides field collected materials, own-rooted plants were propagated from asymptomatic and symptomatic grapevines from Sonoma-1, UCD sources that yielded diseased plants (see results: bench-graft assays) of PN 02A and a healthy source per variety. Also, a healthy source of cv. Thompson seedless table grape was included for testing. To promote rooting, cuttings were processed as described in handling bench-grafts (see above) and plants potted in soil and grown in a greenhouse. In 2009, TNA was extracted from all greenhouse explants and molecularly assayed.

RESULTS

Disease symptoms and vineyard surveys. Surveys of vineyard blocks planted with several clones of PN in Napa County contained scattered grapevines with leaves that were solid red in color (Fig. 1A and 1B). Trunk girths of red-leafed grapevines appeared larger in comparison to those of subtending rootstocks accompanied by scion overgrowths immediately above the scion-rootstock junction. The trunk-union sections of diseased, but not healthy, grapevines showed necrotic tissues at the scion-rootstock junction (Fig. 1C). Examina-

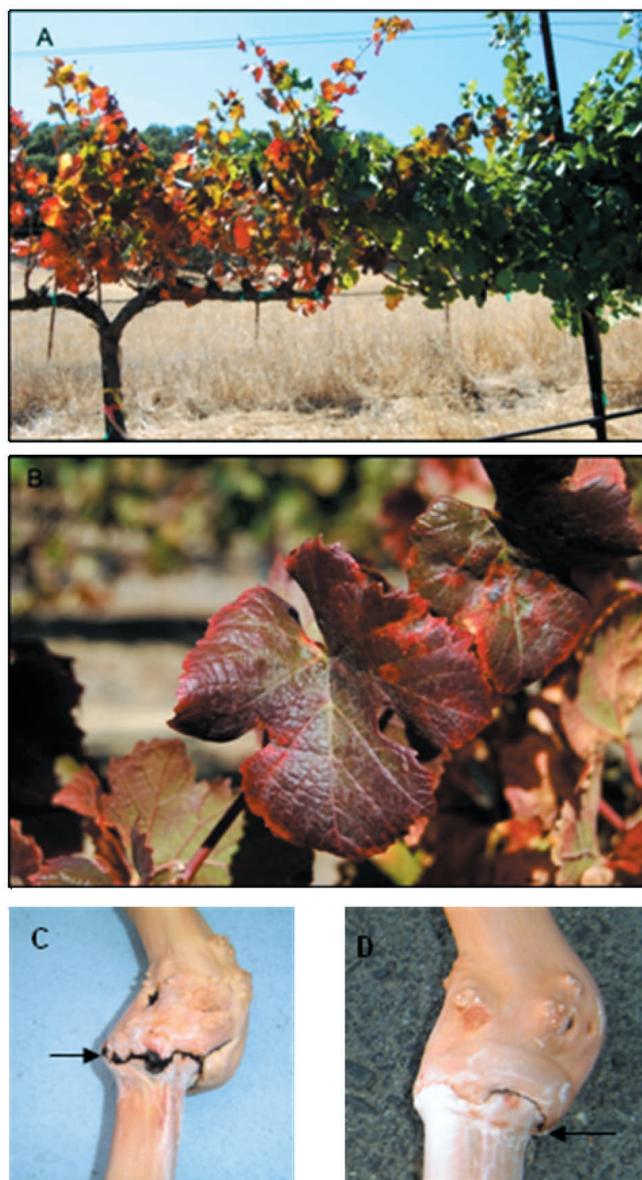


Fig. 1. Canopy and trunk symptoms of grapevine necrotic union disease in early autumn at Sonoma-1 vineyard. Plates A and B, canopy symptoms on cv. Pinot noir 02A; (A) diseased (left) and healthy grapevine (right); (B) close-up view of red leaf. Plate C and D, trunks of cv. Pinot noir 02A showing necrotic union on trunks completely girdled (C) and partially girdled (D). Arrows pointed at graft unions.

tion of roots and ancillary plant parts indicated absence of wood marks, cankers, or bark abnormality that may have had contributed to a girdling event.

One specimen collection, comprised of three trunk pairs of diseased grapevines and neighboring asymptomatic ones, was examined and revealed diseased trunks with necrotic unions while unions of two asymptomatic neighbors lacked similar markings. However, the third specimen exhibited necrotic tissues that encircled half of the scion-rootstock junction (Fig. 1D).

Following surveys in Napa county, other 110R-rooted vineyards in Yolo, Monterey, and Sonoma counties were inspected and found to contain declining grapevines with either red (among PN clones) or yellow (cvs Chardonnay and Pinot gris) canopies and disease diagnosed as GNU. Graft unions of diseased white fruit varieties were necrotic. Incidence of disease in surveyed vineyards ranged from 2 to 45% (Table 1).

In the Sonoma-1 vineyard, the disease incidence was 2.1% in 2004 and rose to 21.9% by 2009 (Fig. 2). Annual surveys also revealed that some grapevines scored previously as symptomless had developed acute disease symptoms (full red canopies with normal grape clusters) by mid-summer of the next growing season. A year later, acute symptomatic grapevines progressed into the chronic disease stage, i.e., stunted shoots and straggly fruit clusters. In Sonoma-1 site, the lower 25% of rows with PN 02A/110R were extended with grapevines of PN 02A propagated on rootstock 3309C. This portion of the vineyard remained free of disease symptoms during the years surveyed.

Biological assays on herbaceous and woody hosts.

All leaf extracts, prepared from symptomatic and asymptomatic tissues failed to incite virus-like symptoms in sap-inoculated herbaceous hosts. In contrast, test plants inoculated with CMV developed typical virus symptoms.

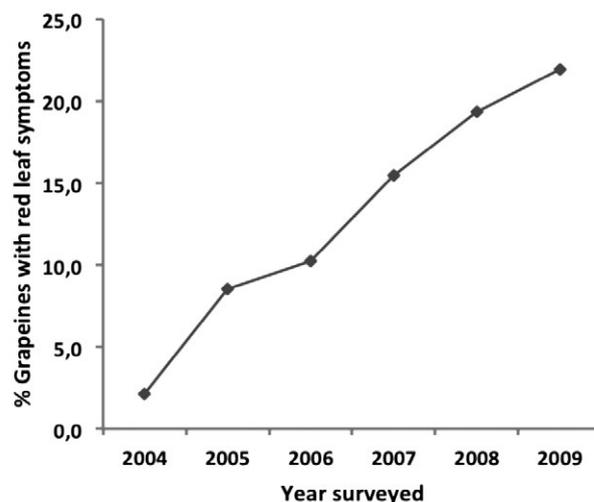


Fig. 2. Cumulative disease incidence of grapevine necrotic union at Sonoma-1 vineyard planted with cv. Pinot noir 02A on 110R. Each year, positions of new grapevines with red leaves were recorded in a sub-block consisting of 8 rows x 80-85 grapevines (total 664 vines) and added to previous year totals.

On woody hosts, bud-chip grafts completed in 2005 (on test plants of CS-8 scions on 10 rootstocks) and 2006 (Chardonnay 04 on six rootstocks) had one to six viable bud-chips per test plant per collection after 30 days post inoculations. Overall, percentages of live bud-chips were 52% (258 live buds/498 total buds) for 2005 assays and 56% (192/340) for 2006 assays. On 110R test plants, percentages of live bud-chips were 60% (58/96) and 52% (30/54) for 2005 and 2006 assays, respectively. No disease symptoms developed in any of the graft-inoculated test plants during the three years of observations. Graft unions of 110R-test plants were normal in appearance.

Evaluation of bench-grafts totaled 300 scion-rootstock combinations. Of these, 19 finished plants in the field developed GNU symptoms and these plants came

Table 1. Incidence of grapevine necrotic union disease in 110R rooted vineyards in Napa, Monterey, Sonoma, and Yolo counties of California.

Year surveyed	County	Varieties and clones	Number of grapevines	Number diseased (%)
2004	Napa	Pinot noir 02A	615	72 (11.7)
		Pinot noir 667	417	21 (5.0)
		Pinot noir 777	299	135 (45.2)
		Pinot noir UCD 04	200	48 (24.0)
2006	Sonoma-1 [†]	Pinot noir 02A	664	14 (2.1)
		Chardonnay 04	50	1 (2.0)
2008	Monterey	Pinot gris 152	200	6 (3.0)
		Pinot noir UCD 04	400	12 (3.0)
		Pinot noir UCD 04	401	38 (9.5)

[†]Sonoma-1 and Sonoma-2 are different properties

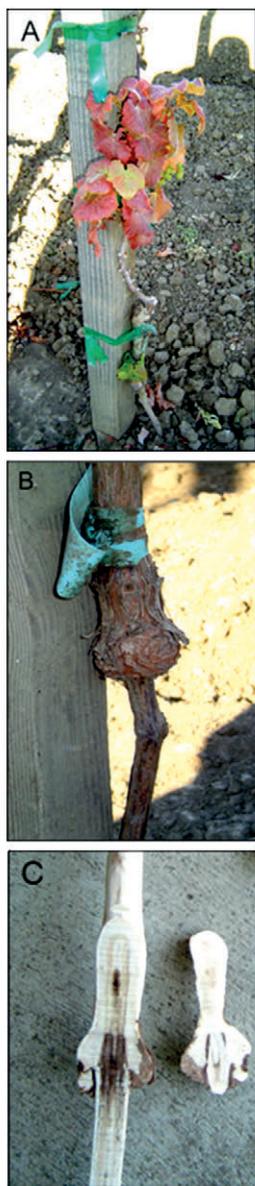


Fig. 3. Bench-grafts of diseased cv. Pinot noir 02A on 110R rootstock and symptoms of grapevine necrotic union disease: (A) red leaf; (B) swollen scion at graft union; and (C) internal view of diseased graft union.

from 11 UCD sources. Specifically, eight collections of PN 02A were infective: four sources yielded one diseased plant each, two sources yielded two diseased plants each, and two sources yielded three diseased plants each. Also, two collections of PN 91 yielded two diseased plants each and one Chardonnay 04 source yielded one diseased plant. All diseased plants exhibited red (PN clones) or yellow (Chardonnay 04) leaves, poor shoot growth, and necrotic unions (Fig. 3). None of the other bench-grafts with field collections on rootstock 110R, own-rooted scions or rootstock Kober 5BB developed GNU-associated symptoms when bench-grafted onto rootstock 3309C.

Molecular assays. With the exceptions of UCD sources of PN 02A and Thompson Seedless, which tested negative to all viruses with the primers used, TNA preparations from other collections, irrespective of disease status, tested positive for one or more viruses (Table 2). All preparations were negative for leafroll-associated viruses, nepoviruses, vitiviruses, phytoplasmas and prokaryotes. Standard positive and negative controls reacted in the expected manner.

DISCUSSION

The occurrence of GNU-diseased vineyards indicates an emerging disease in vineyards devoted to wine grape production in California. Diseased vineyards were identified in coastal counties of Monterey and Sonoma and inland counties of Napa and Yolo with disease incidence ranging from 2 to 45% (Table 1).

Evidence for secondary spread of a putative infectious agent was based on field data collected during repeated surveys of Sonoma-1 beginning in 2004, in an eight years old vineyard. Initial disease incidence was 2.1%, which gradually increased to 21.9% by 2009 (Fig. 2). During this period, detailed mapping of diseased grapevines illustrated the rapid progression of disease symptom development. Several grapevines scored as symptomless progressed to the acute disease stage in the next growing season, and then, advanced to the chronic disease stage which was evident two years later.

Another line of evidence for ongoing disease spread was obtained after examining three pairs of trunk unions comprised of GNU-diseased and asymptomatic neighboring grapevines. Necrotic tissues had completely encircled the unions of diseased specimens, whereas trunks of two asymptomatic ones had healthy unions, but the third specimen had necrotic tissues that encircled half of the union (Fig. 1D), which was suggestive of a recent infection event. It would appear that complete encirclement by necrotic tissues along the unions, i.e. trunks with large girths, would require more than one year incubation post infection. This is due, in part, to the hypersensitive response of 110R rootstock, resulting in cell death upon infection to impede rapid movement of the causal agent along the graft union.

The red canopy symptoms of GNU disease were similar to those described for cv. Redglobe with grapevine stem lesion disease on the virus sensitive rootstocks 3309C, Kober 5BB, 1616C, 1103P, and Teleki 5C (Uyemoto *et al.*, 2001) or grapevine stem necrosis-distortion of clone PN 23 on rootstock 3309C (Lima *et al.*, 2009). Both causal agents incite necrotic reactions in the rootstocks extending beyond graft unions (Uyemoto *et al.*, 2001). With GNU, however, tissue necrosis was concentrated (limited) at the scion-rootstock junction, a reaction similarly reported for walnut blackline disease in-

Table 2. Molecular assays by RT-PCR for viruses and prokaryotes in grapevines asymptomatic and symptomatic for grapevine necrotic union disease. Total nucleic acid preparations were of field or own-rooted explant collected from vineyards: Napa, Sonoma-1, and UCD.

Vineyards and grape clones	Viruses ¹				
	GFkV	GLRaV-7	GRSPaV	GSyV-1	GRVfV
Napa					
Pinot noir (PN) 02A ²	-	-	+	-	-
PN 02A ³	+	-	+	-	+
PN 667 ²	-	-	+	+	-
PN 777 vine-1 ²	+	-	+	+	-
PN 777 vine-2 ²	+	-	+	-	-
PN 777 vine-3 ²	-	-	+	+	-
PN 777 vine-4 ³	+	-	+	-	-
PN UCD04 ²	+	-	+	+	-
PN UCD04 ³	+	-	+	-	-
Sonoma-1					
PN 02A vine-1 ²	-	-	+	-	-
PN 02A vine-2 ²	-	-	+	+	-
PN 02A vine-3 ³	+	-	+	-	-
UCD					
PN 02A (five vines) ²	-	-	-	-	-
PN 02A ³	-	-	-	-	-
Thompson seedless ³	-	-	-	-	-

¹*Grapevine fleck virus* (GFkV); Grapevine leafroll-associated virus -7 (GLRaV-7); *Grapevine rupestris stem pitting-associated virus* (GRSPaV); Grapevine Syrah virus-1 (GSyV-1), *Grapevine rupestris vein feathering virus* (GRVfV). All samples tested negative for *Grapevine leafroll-associated virus* (GLRaV) -1, -2, -4, -5, -9, GLRaV-2RG, and Grapevine leafroll-associated Carnelian virus, *Grapevine virus A*, *Grapevine virus B* and *Grapevine virus D*, *Arabis mosaic virus*, *Grapevine fanleaf virus*, *Tobacco ringspot virus*, *Tomato ringspot virus*, and *Grapevine vein feathering virus* in RT-PCR analysis, and phytoplasmas and *Xylella fastidiosa* in PCR analysis.

²Sources exhibiting GNU symptoms and sources identified as GNU-positives by bench-graft assays.

³Asymptomatic grapevine sources.

cited by *Cherry leafroll virus* (CLRV) (Mircetich and Rowhani, 1984) and *Prunus brown line* incited by *Tomato ringspot virus* (ToRSV) (Mircetich and Hoy, 1981).

Genetic graft-incompatibility among *Vitis* species is, at best, a rarity and the combination of PN clones and 110R rootstock is considered highly compatible (Gökbayrak *et al.*, 2007). Previously, virus-induced graft incompatibility in grapevines involved ToRSV infections of cv. Seyval blanc scions grafted on cvs Baco noir and White Riesling on 3309C rootstocks (Uyemoto *et al.*, 1978). Both Baco noir and 3309C are susceptible to systemic infection by the nematode-vectored ToRSV while both scion cultivars are putatively hypersensitive to the virus. In France and Italy, GLRaV-2 was associated with graft incompatibility of Chardonnay accessions on rootstock Kober 5BB (Greif *et al.*, 1995). In California, a stem lesion disease was observed in field trials of table grape cv. Redglobe on the rootstocks 3309C, 1103

Paulsen, Kober 5BB, and Teleki 5C (Uyemoto *et al.*, 2000; Uyemoto and Rowhani, 2003). Subsequently, a genetically distinct strain of GLRaV-2 was found associated with this disease, and designated GLRaV-2RG (Rowhani *et al.*, 2000; Uyemoto *et al.*, 2001). Hence, a search for the infectious agent for GNU-disease was undertaken.

Bioassays onto herbaceous host plants via sap inoculations and grapevine indicators via bud-chip grafts were inconclusive. On grapevine test plants, failure of bud-chip assays suggested either extremely low pathogen titer or an irregular distribution in symptomatic grapevines. However, bench-grafts made with canes collected from UCD vineyard produced symptoms of GNU. The collection was comprised of PN 02A, PN 91, and Chardonnay 04 which were asymptomatic on their own-roots or on Kober 5BB. The latter collections were not used in bud-chip assays.

Failure of bench-grafts using canes from symptomatic

grapevines was, perhaps, due to the absence of the etiological agent in the canes collected. This scenario likely resulted when dormant grapevines, pruned to two-bud spurs, produced cane growths “free” of the pathogen. Onset of acute and chronic disease stages are the consequences of “girdled” necrotic union. Apparently, within symptomatic grapevines on 110R rootstock, lateral movement of the putative pathogen is constrained. A similar rapid vertical, albeit slower lateral, spread of CLRV occurred in English walnut trees (Mircetich *et al.*, 1998). In contrast, the putative GNU incitant in asymptomatic grapevines, when self-rooted or grafted on a permissive rootstock like Kober 5BB, is not limited in its systemic spread throughout the grapevine.

Symptomless virus infections in grapevine have been known for a long time (Greif *et al.*, 1995; Saayman and Lambrechts, 1993). A strain of GLRaV-2, asymptomatic in cv. Chardonnay exhibited graft incompatibility with Kober 5BB (Greif *et al.*, 1995), and the other associated with graft incompatibility of cv. Red Globe was asymptomatic not only in cv. Red globe, but also in Cabernet sauvignon and Cabernet franc, which are standard leafroll disease indicators used in grapevine clean stock programs (Uyemoto *et al.*, 2001). Even though red leaf symptoms were clearly different from the leafroll disease, all grapevine samples in the present investigation were, nonetheless, tested for GLRaV2-RG. Incidentally, in New Zealand, an isolate of GLRaV-2 was associated with a red leaf disease in grapevines of cv. Merlot 481 on rootstocks 3306C and Riparia Gloire, but not on rootstocks 3309C and 101-14 Mtg (Bonfiglioli *et al.*, 2003). This GLRaV-2 isolate was 99% identical to GLRaV-2RG. The apparent lack of response of 3309C rootstock to Merlot 481 scions is indicative of a wide diversity in pathogenicity among strains of GLRaV-2. This can be a challenge for grapevine registration and certification program (Rowhani *et al.*, 2005). In a recent study, Bertazzon *et al.* (2010) analyzed the genetic variability and pathological properties of GLRaV-2 isolates and found that they belonged to three groups with distinct pathological properties; one causing mild leafroll symptoms but unable to induce graft incompatibility, one that caused graft incompatibility without causing leafroll symptoms, and the last that caused strong leafroll symptoms and graft incompatibility. Currently, leafroll-associated viruses other than GLRaV-2, are not known to cause graft incompatibility.

The rootstock 110R is widely planted in California, in European countries, Israel, and North Africa. It is the third most used rootstock in France (Hellman, 2007); highly resistant to phylloxera, well suited in arid regions and on lime soils (Southey and Fouché, 1992). Because of its commercial popularity and value, it is essential to develop effective management strategies to prevent GNU. Hence, identification of the etiological agent is pivotal to develop rapid and reliable detection assays to identify

clean propagation sources. In the meantime, it would be advisable to maintain grapevines of PN clones on 110R, a self-indicator of GNU-causal agent.

Our attempts to detect a putative GNU agent by RT-PCR analysis has served to remove from consideration several of the known grapevine viruses, phytoplasmas, and *Xylella*. It is likely that a novel virus is associated with GNU and unraveling the causal agent is best served by next generation sequencing as demonstrated in some of the recent studies (Al Rwahnih *et al.*, 2009; Coetzee *et al.*, 2010; Kreuze *et al.*, 2009; Pantaleo *et al.*, 2009; Zhang *et al.*, 2011).

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Disclaimers

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